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Schematic cross-linking of HEC with glyoxal

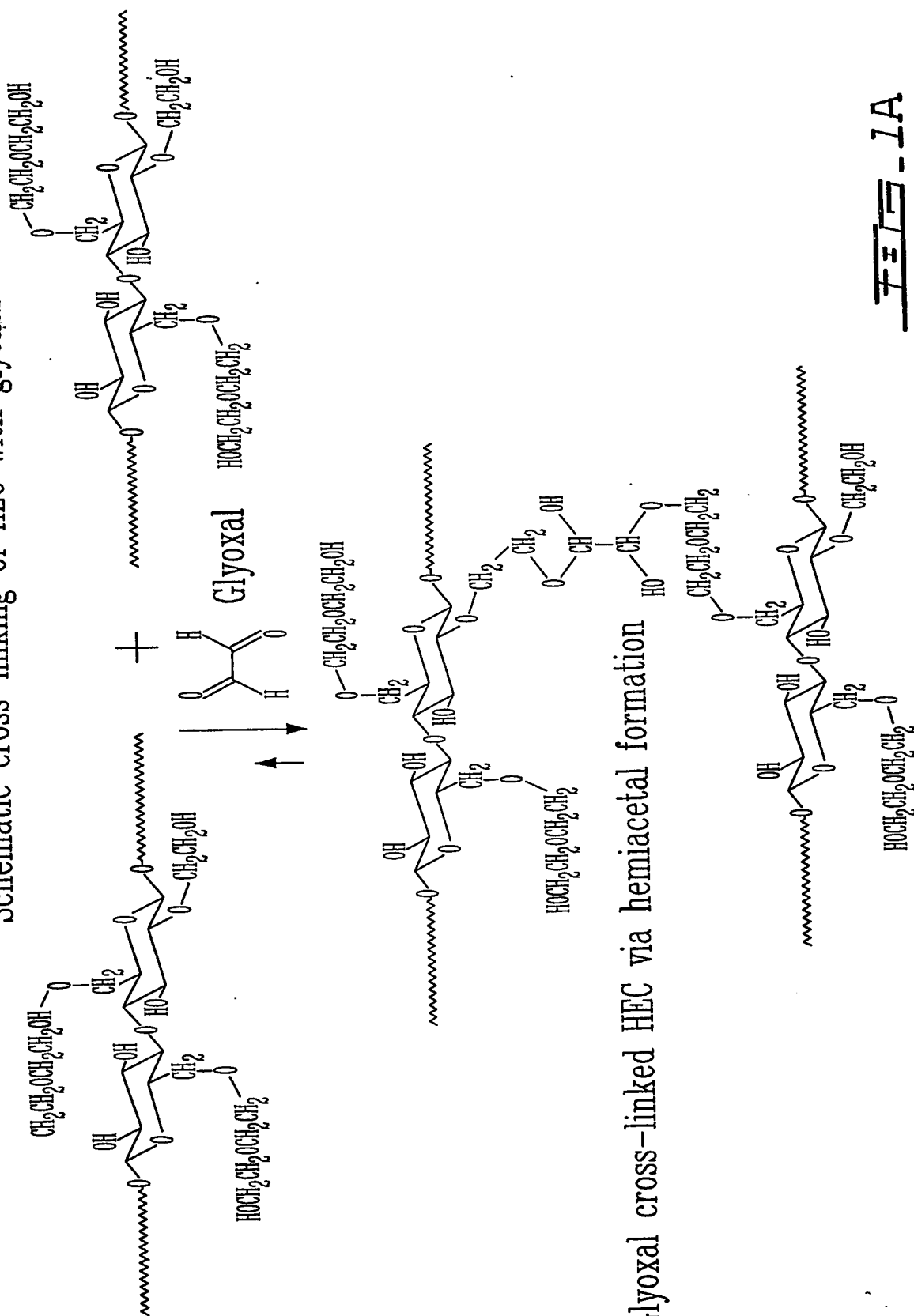
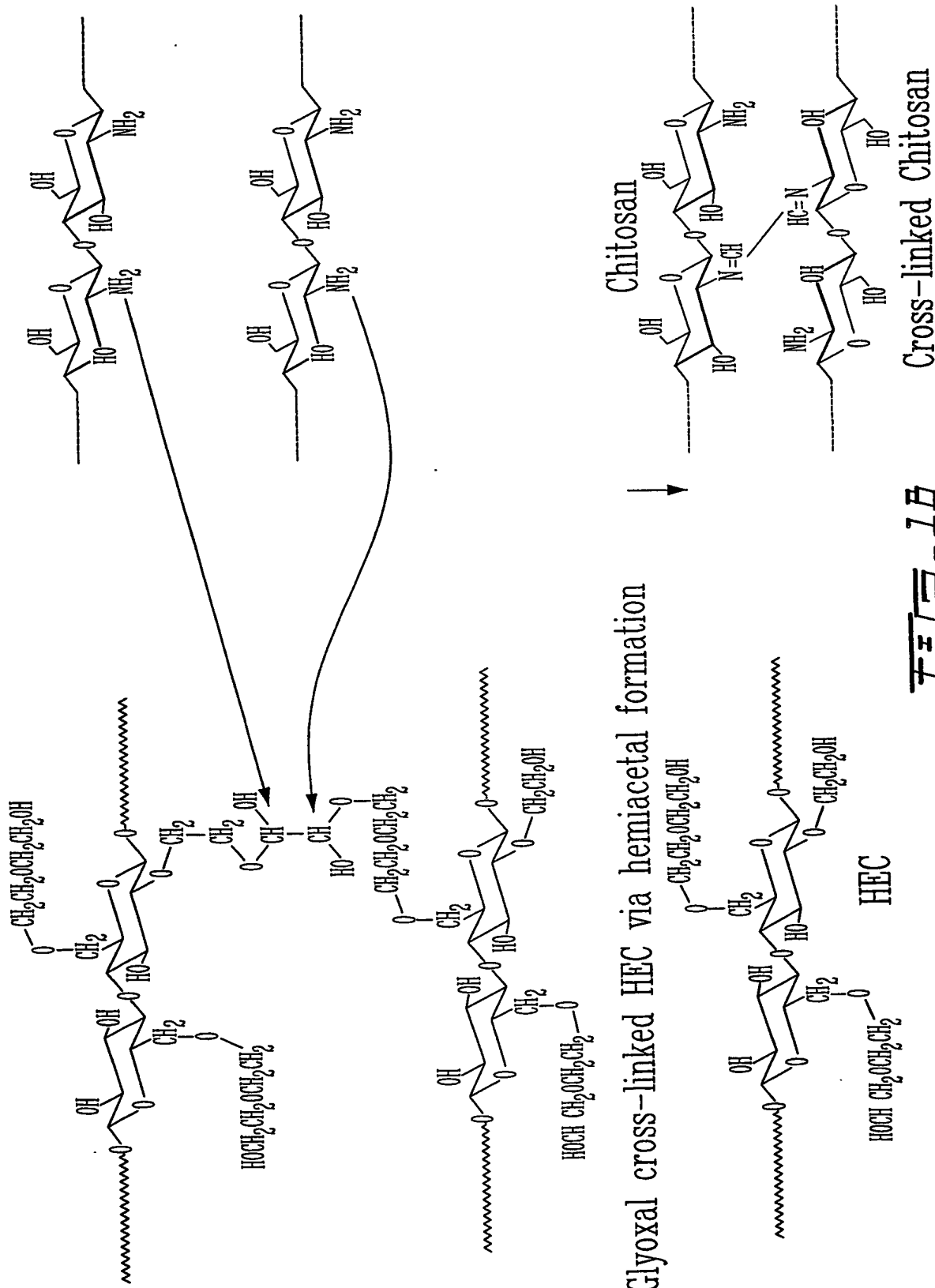
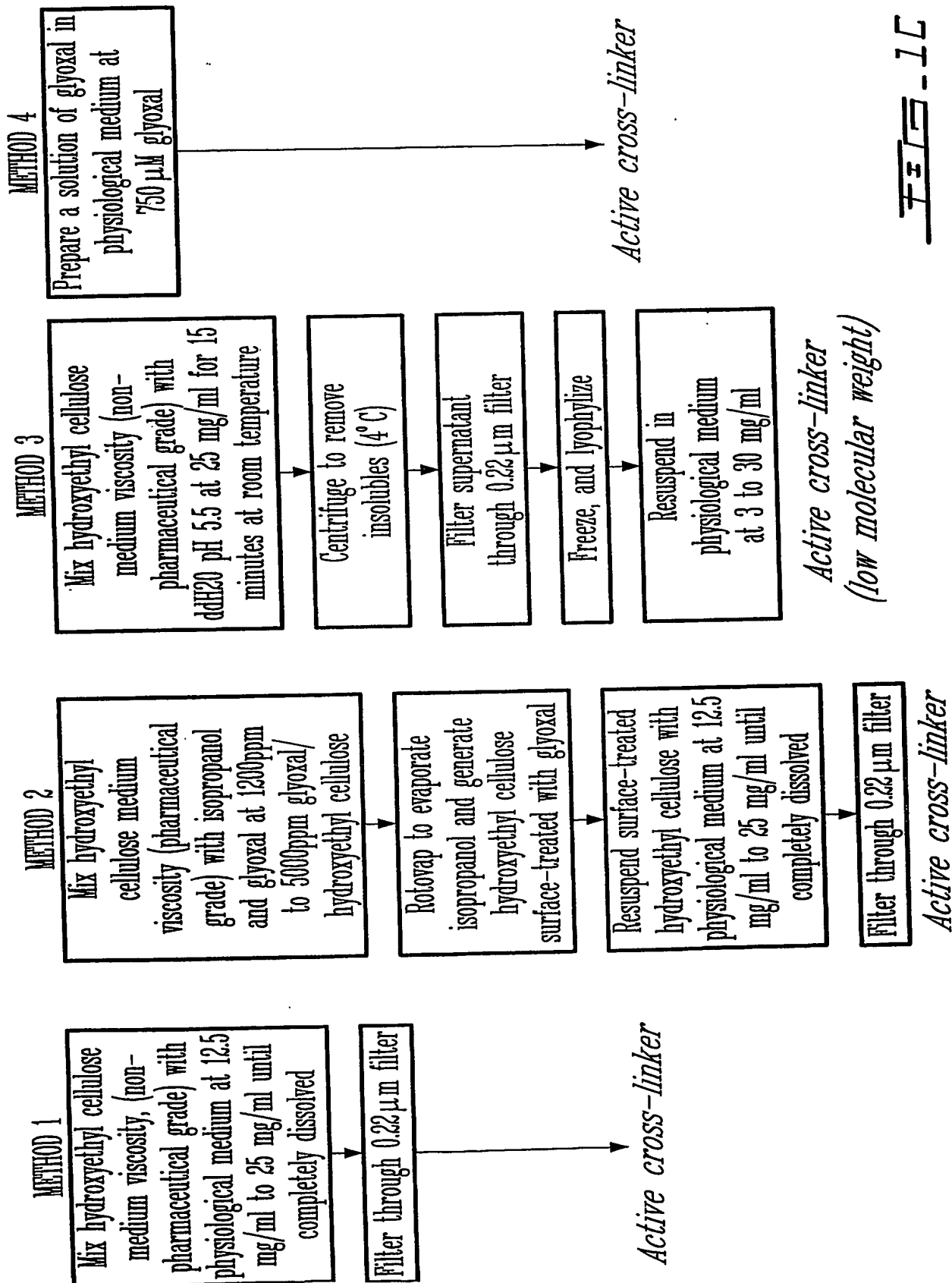


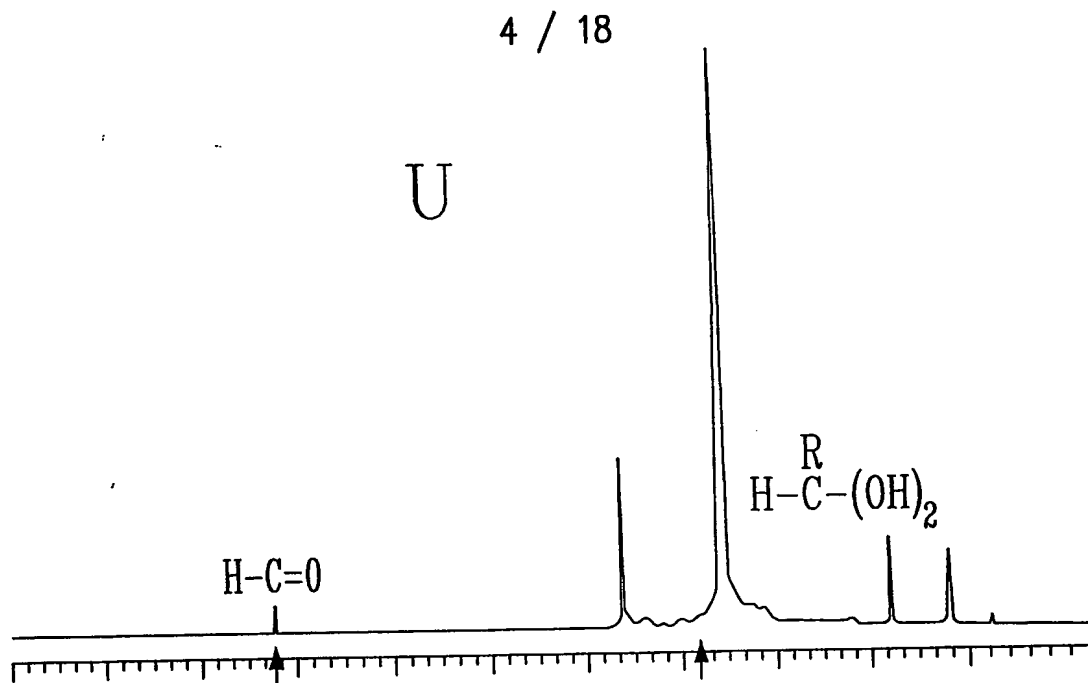
FIG. 1A

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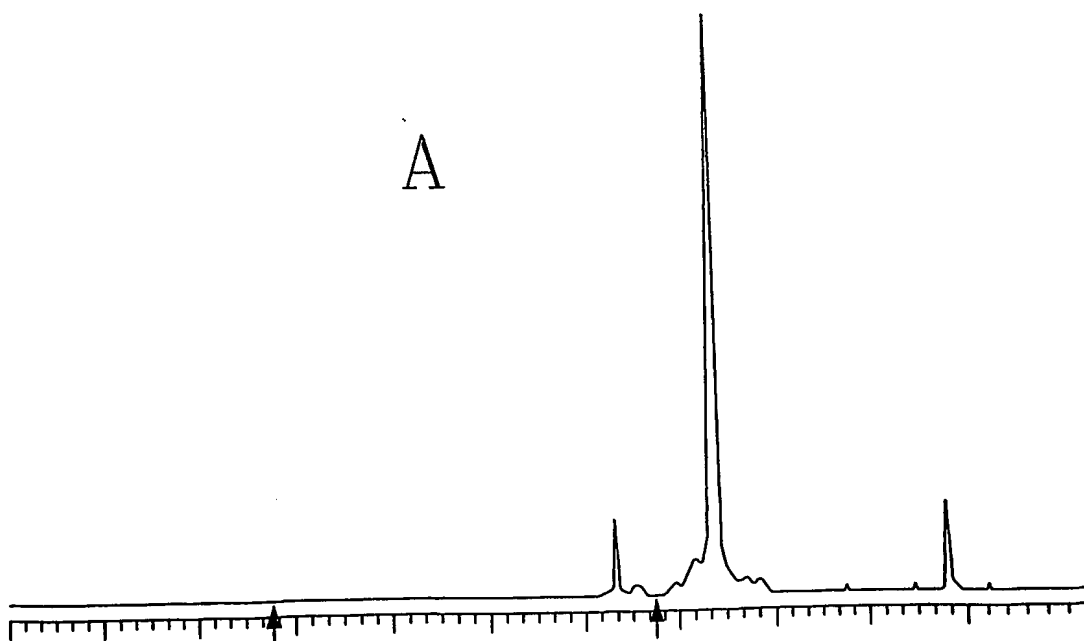
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U: Unfractionated HEC = active

FIG. 2A



A: HEC: Above 1000 Da = inactive

FIG. 2B

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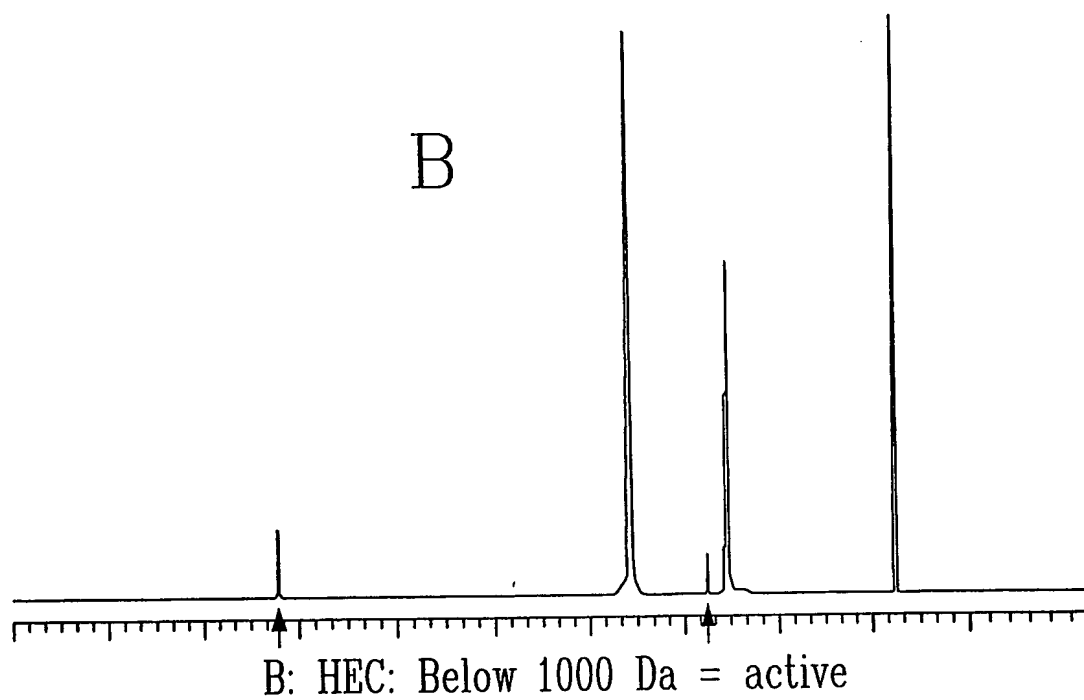


FIG. 2C

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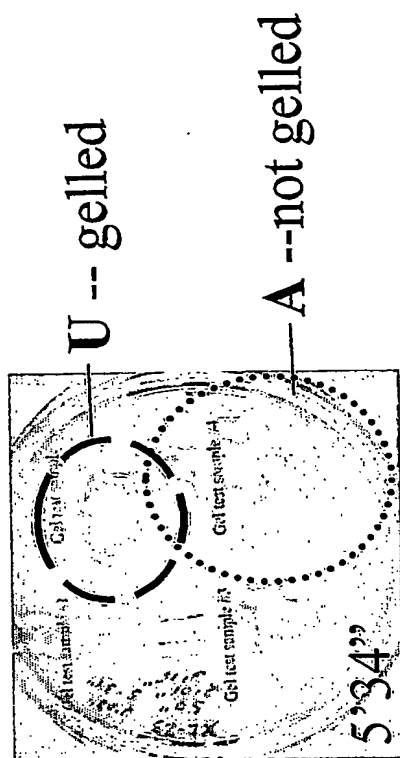


FIG. 2E

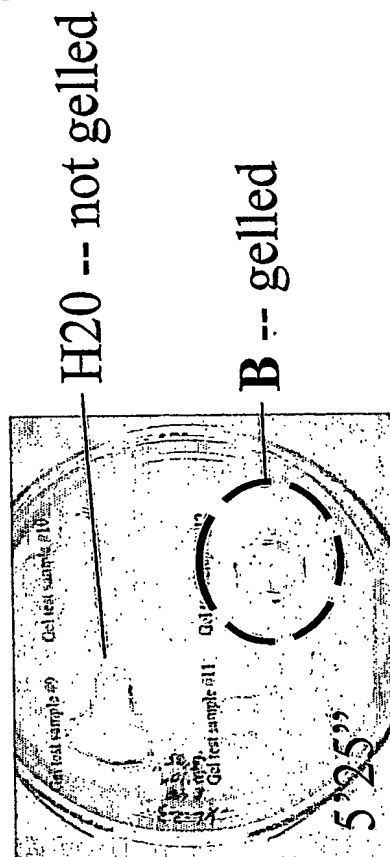


FIG. 2G

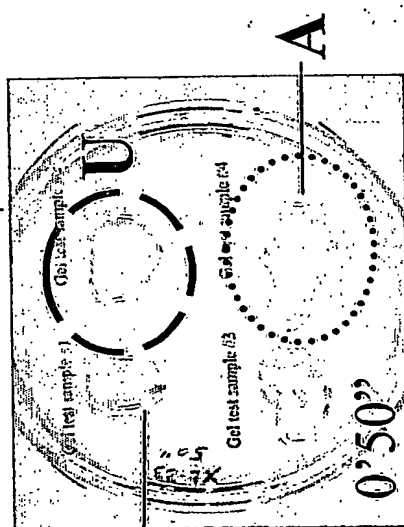


FIG. 2D

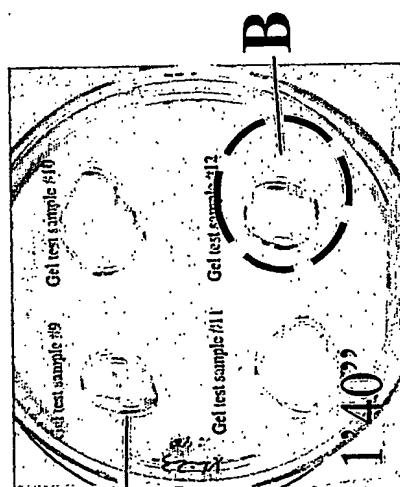


FIG. 2F

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CHITOSAN: HEC/aldehyde GEL TIME

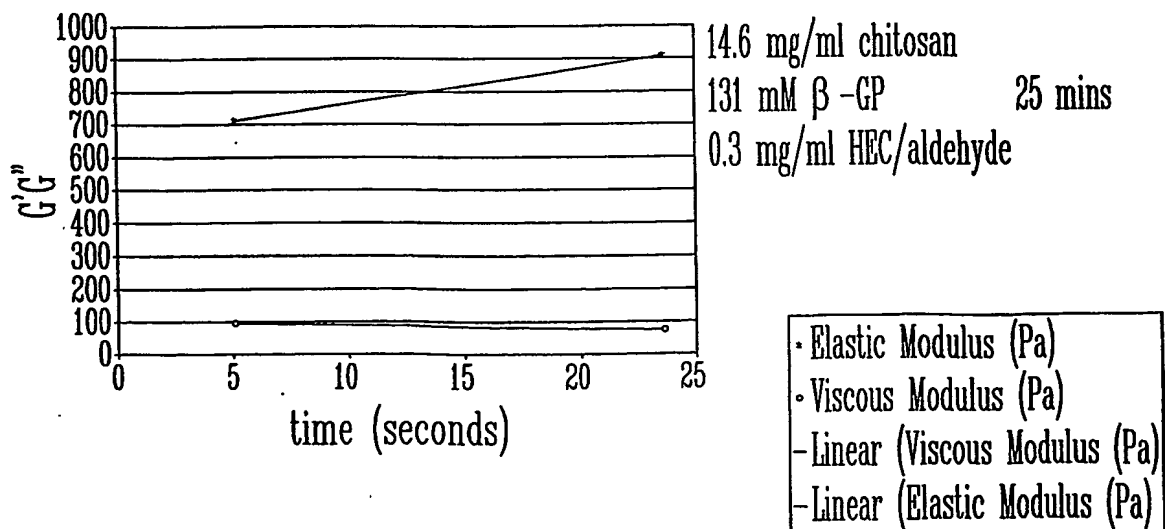
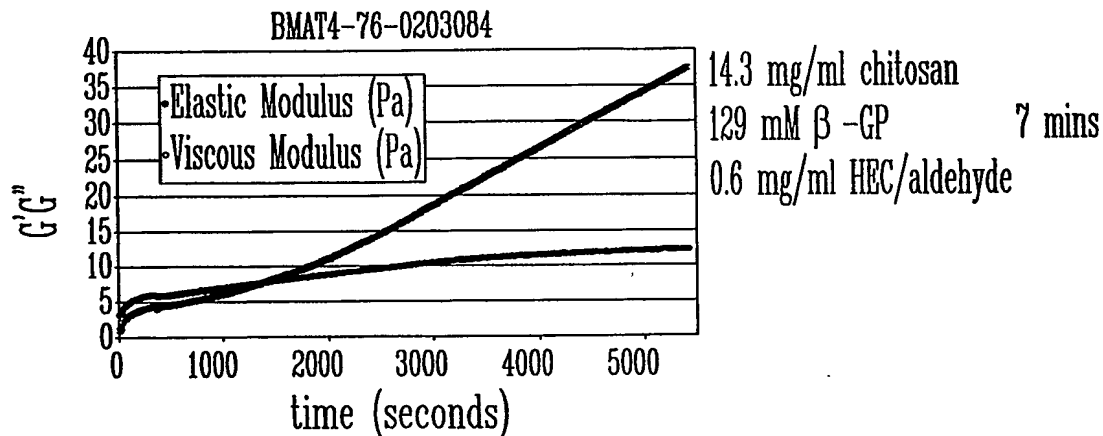
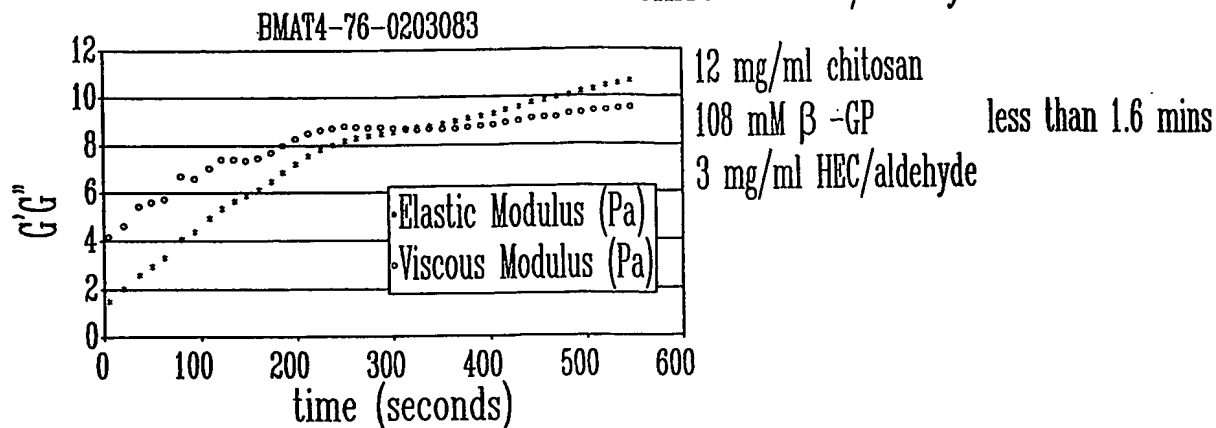


FIG. 3

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PASSAGED BOVINE CHONDROCYTES

CULTURED IN CROSS-LINKER SOLUTION

2 HOURS CULTURE 72 HOURS CULTURE

LIVE (green) DEAD (red)

7E/11 - 4A

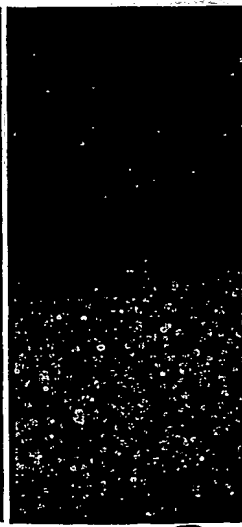
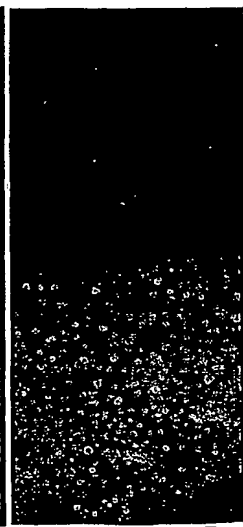
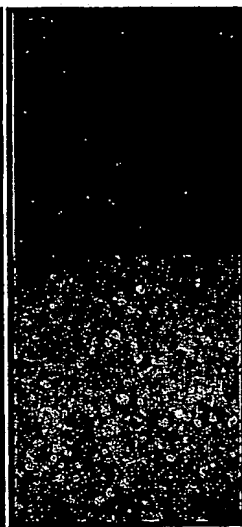
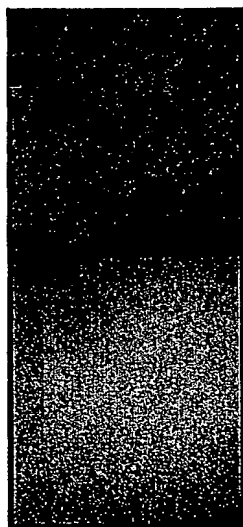
1 DAY POST-  
ENCAPSULATION  
VIABLE CELLS

0.3% H<sub>2</sub>O<sub>2</sub>  
(negative  
control)

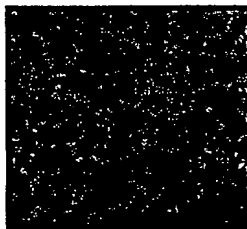
1.3 mM  
glyoxal  
(METHOD 4)

HEC-glyoxal  
(METHOD 2)

HEC-glyoxal  
(METHOD 1)



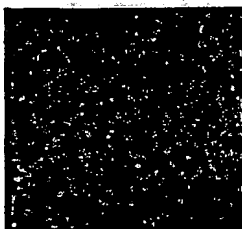
750  $\mu$ M  
[glyoxal]



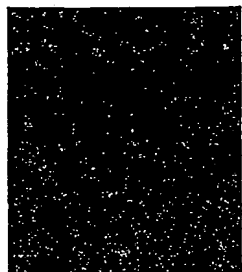
IMMEDIATE  
POST-  
ENCAPSULATION  
VIABLE CELLS

extruded through  
a 26g needle

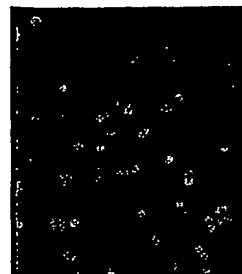
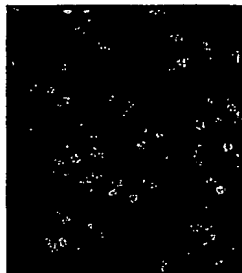
25 mg/ml  
[HEC]



5 mg/ml  
[HEC]<sub>final</sub>



150  $\mu$ M  
[glyoxal]<sub>final</sub>



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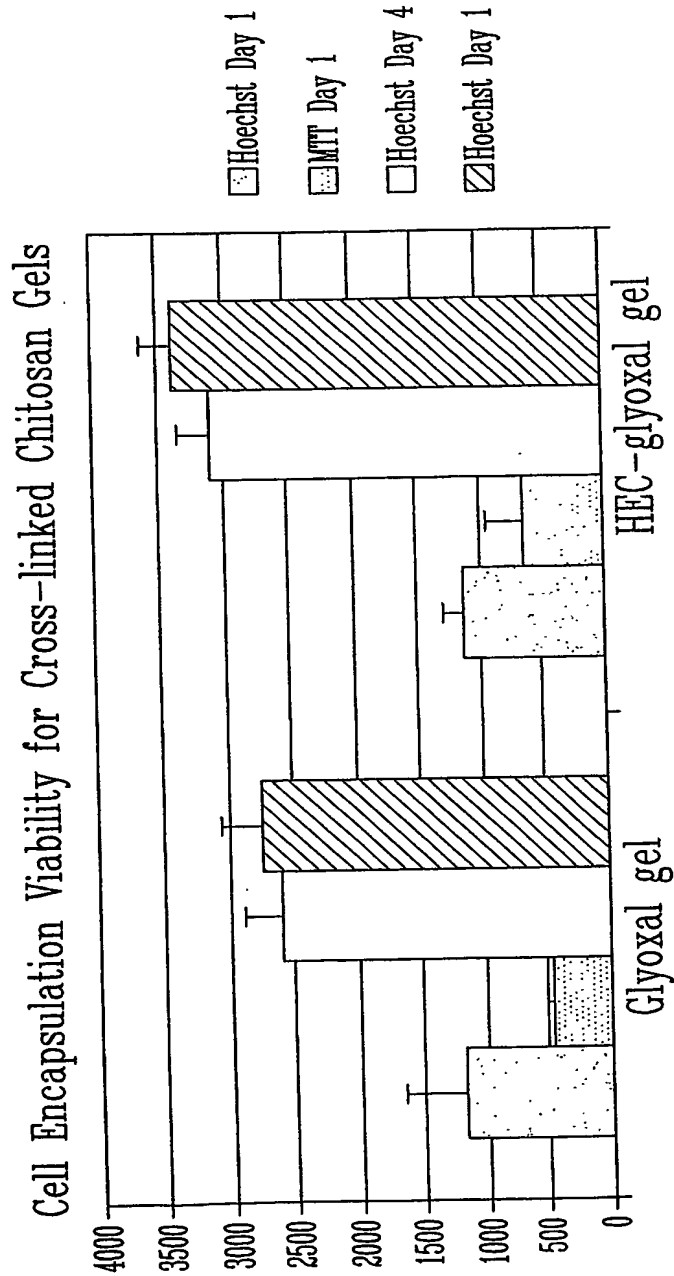


FIG. 4B

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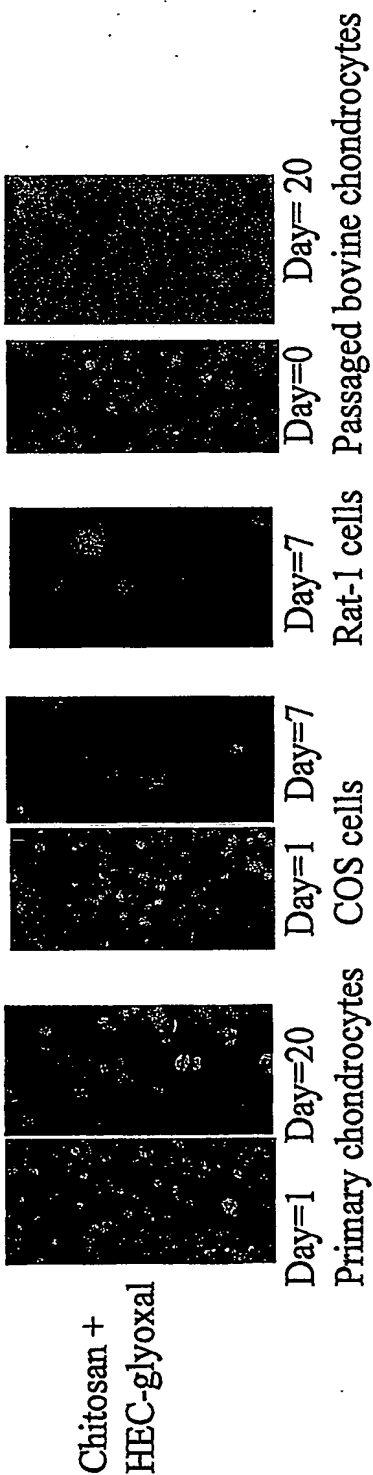


Fig. 5A

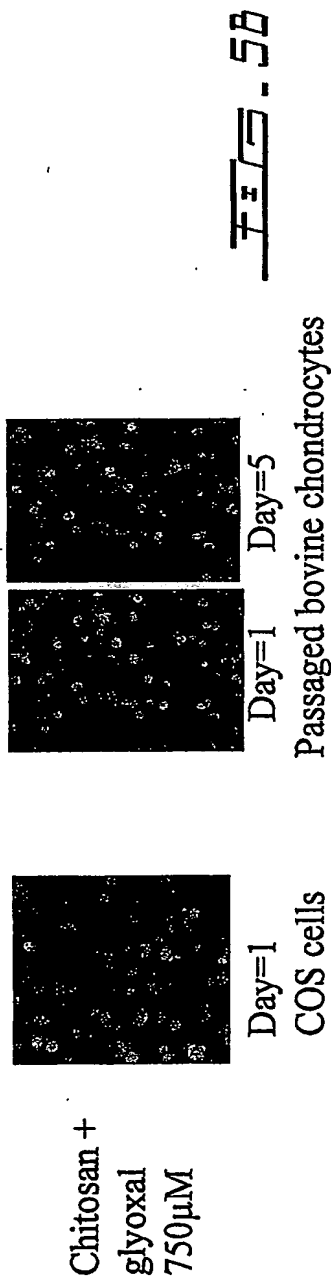


Fig. 5B

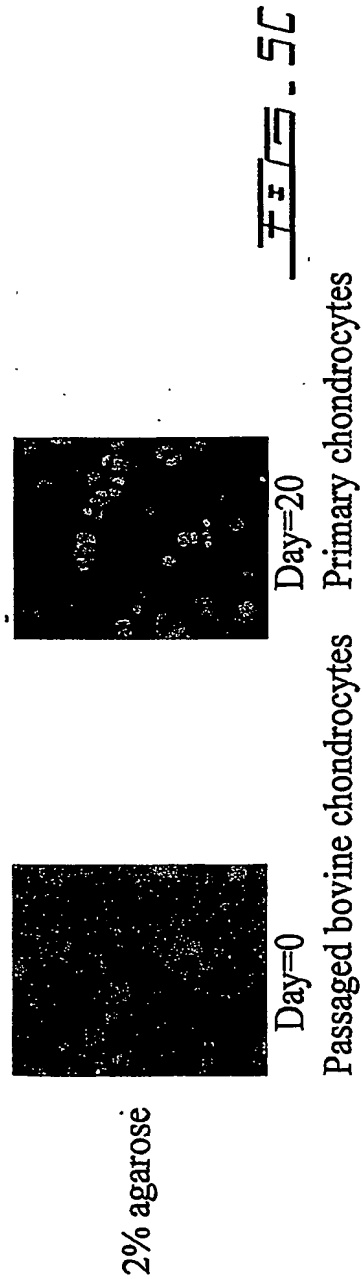


Fig. 5C

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Composition of final gel

	% (w/v)	Mol/L	Final pH (room temp) pH 6.85
Protasan UP CL 213 starting pH 5.5	1.35	0.08	
NaOH		0.018	
Glucosamine	0.38	0.018	
Disodium beta - GP	2.06	0.104	
<b>METHOD 1</b> Fluka Hydroxyethyl cellulose- glyoxal 25 mg/ml original concentration	0.4 (~0.001% glyoxal)	(~ 150µM glyoxal)	

Composition of final gel

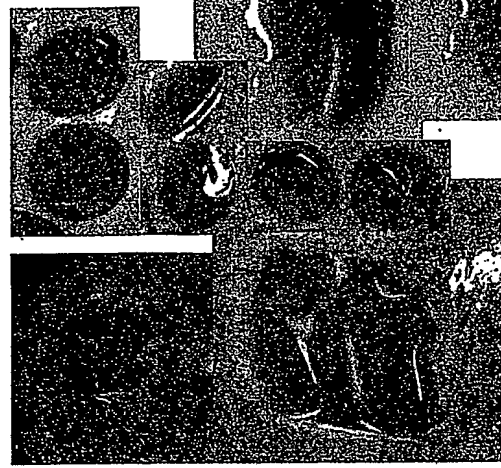
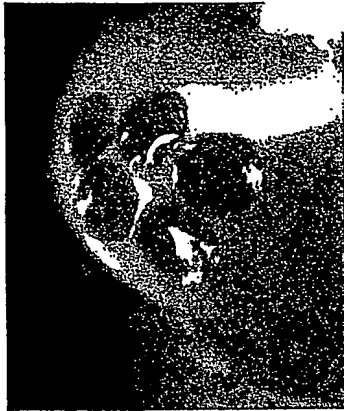
	% (w/v)	Mol/L	Final pH (room temp) pH 6.75
Chitosan 83%DDA in 67 mM HCl starting pH 4.5	1.2	0.073	
Disodium beta - GP	2.4	0.113	
<b>METHOD 2</b> Hydroxyethyl cellulose-2500 ppm glyoxal, 25 mg/ml original concentration	0.4 (~0.001% glyoxal)	(~ 150µM glyoxal)	

Composition of final gel

	% (w/v)	Mol/L	Final pH (room temp) pH 6.75
Chitosan 83%DDA in 67 mM HCl starting pH 4.5	1.2	0.073	
Disodium beta - GP	2.4	0.113	
<b>METHOD 4</b> glyoxal 750µM original concentration	~ 0.001%	~ 150µM	

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Full thickness

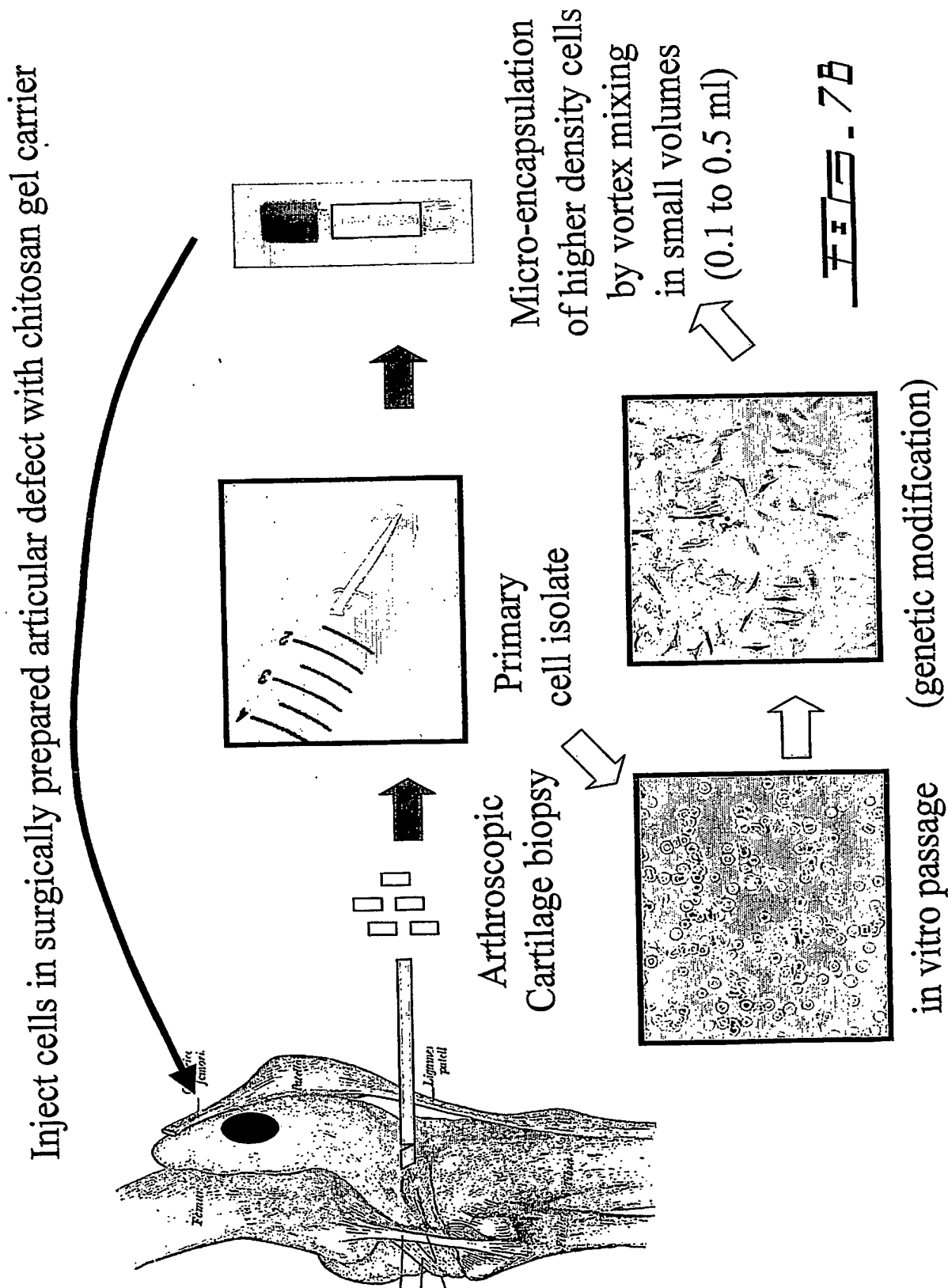


Partial thickness

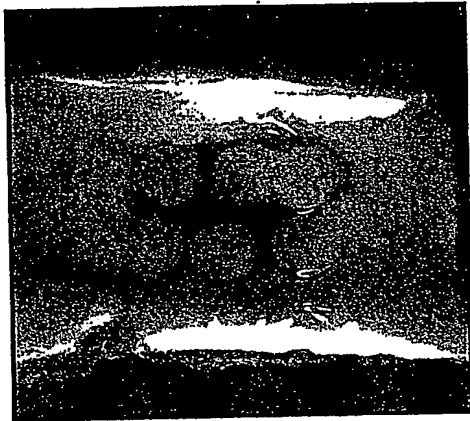


Figs. 7A

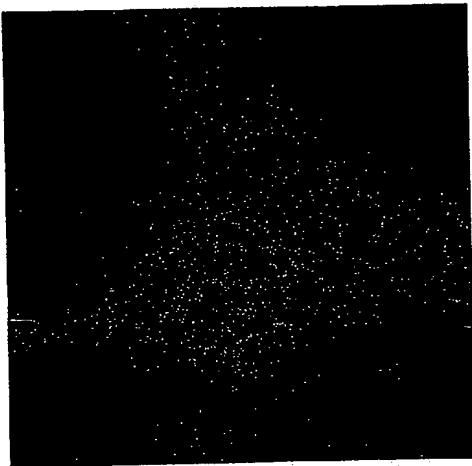
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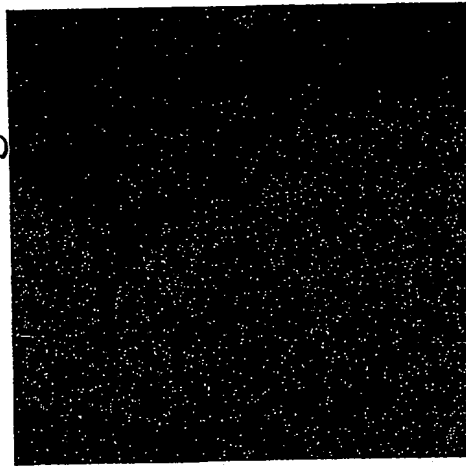
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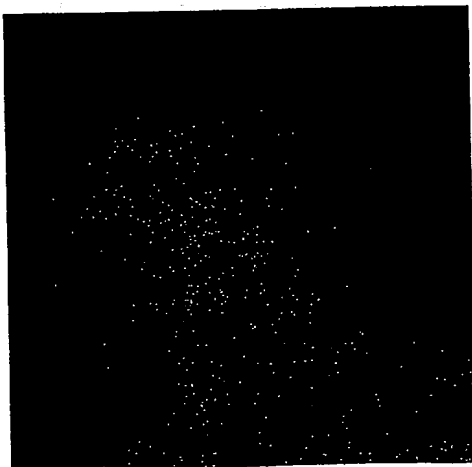
Prepared treated  
porcine defect  
(Hoechst-stained blue  
chondrocytes) loaded  
with cross-linked  
chitosan delivering live  
(Calcein AM stained  
green) primary  
chondrocytes



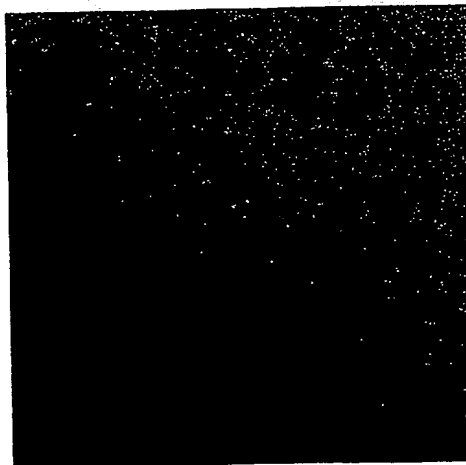
4x mag



20x mag



4x mag



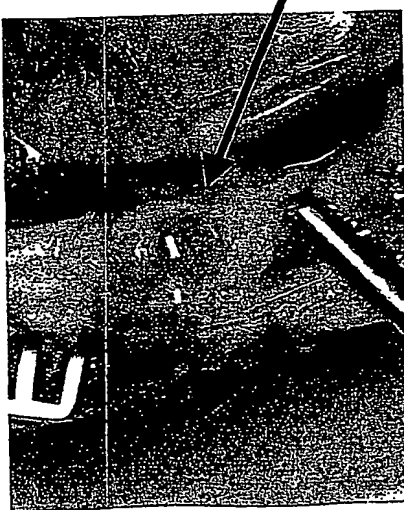
40x mag

Fig. 7C

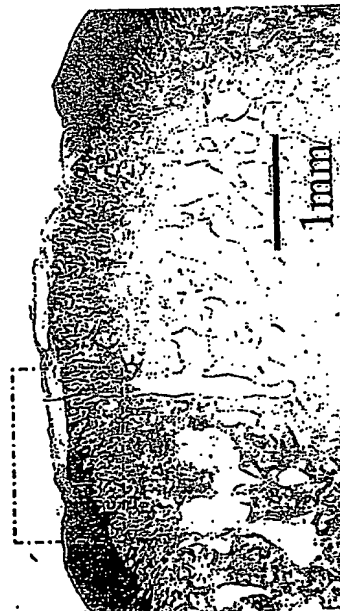
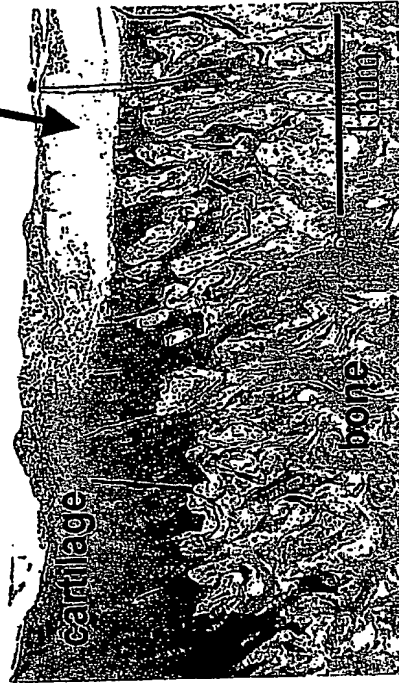
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Surgical implant of  
cross-linked chitosan  
in  
patella and  
femoral-patellar groove



chitosan gel



Patellar Implant after 1 day in vivo

FIG. 8A

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no implant

FIG. 8B

Cross-linked chitosan implant



blood clot

Day 1

chitosan gel

20x

blood clot



chitosan gel

Day 7

10x



New woven bone



2.5X



chitosan gel

Day 32

2.5x

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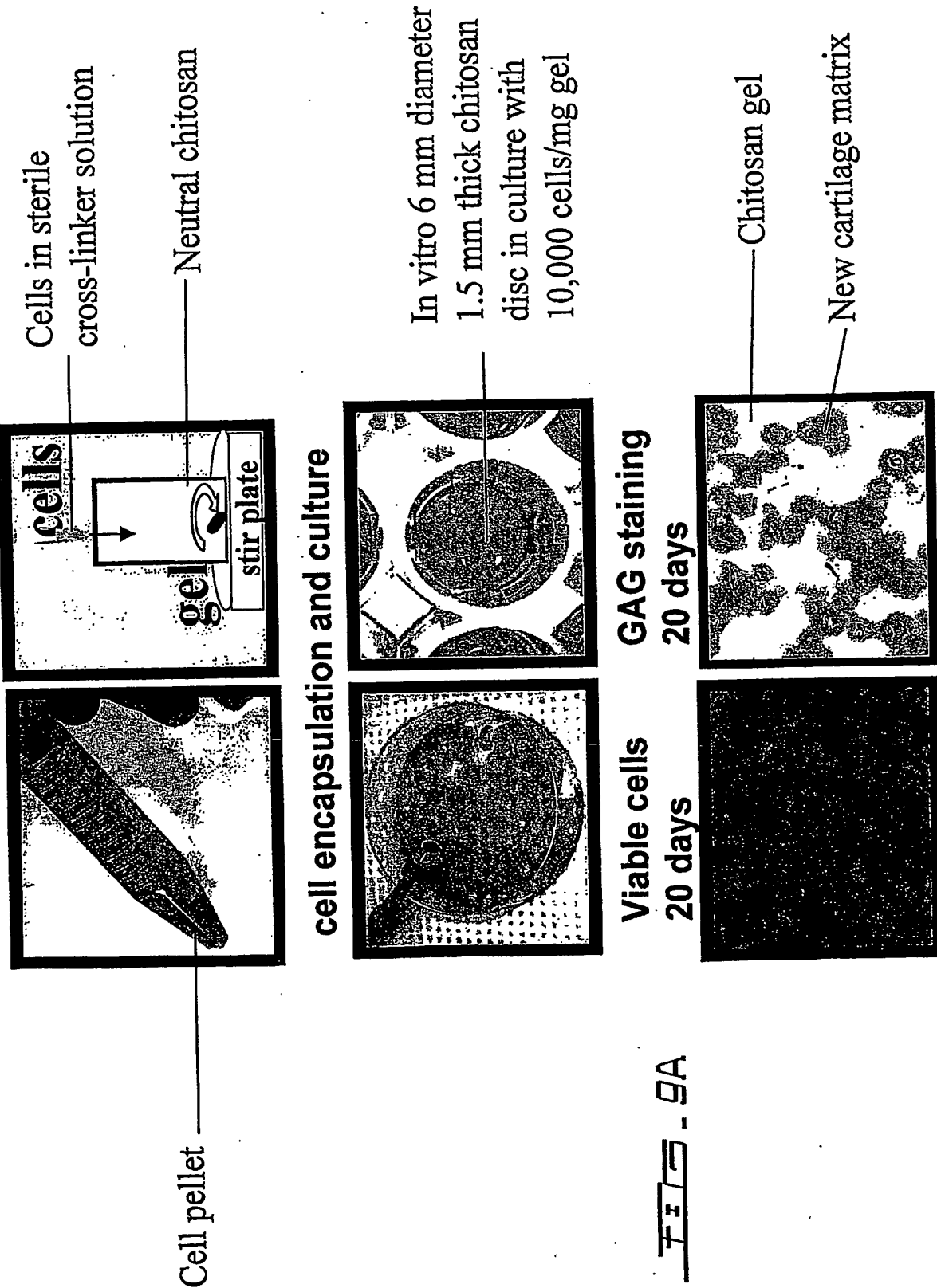
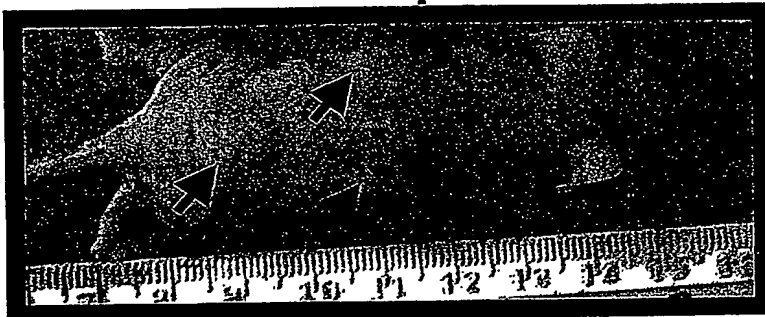


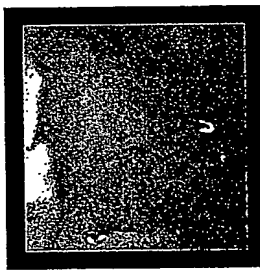
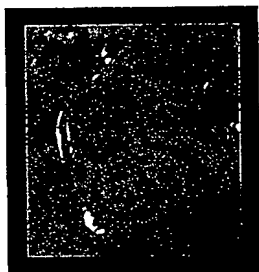
FIG. 9A

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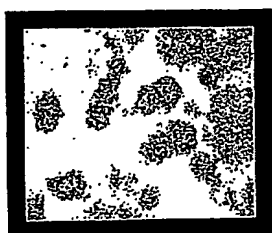
## In Vivo implants



## Dissected implants with chondrocytes



## GAG staining 48 days      63 days



FIS - 9B